Module 6 : Hypersensitivity and immunodeficiency

Lecture 35: Hypersensitivity (Part I)

Hypersensitivity diseases

Diseases or ailments caused by impaired immune responses are called hypersensitivity disorders.

35.1 Causes of hypersensitivity diseases

Autoimmunity

Reactions against microbes

Reactions against environmental antigens

Autoimmunity

It may be defined as the failure of normal process of an individual to distinguish between self and non-self i.e when the individual fails to recognize its own parts as self and develops an immune response against its own cells and tissues. Diseases that occur because of autoimmunity are called as autoimmune diseases.

Reactions against microbes

Reactions against persistent microbial agent may occur in the form of T-cell response. Tuberculosis, inflammatory bowel disease and viral hepatitis are some of the related conditions.

Reactions against environmental antigens

It does not occur in majority of the population but very less percentage of the individuals may show reaction against some harmless environmental products. As a result of allergy such patients generate immunoglobulin E (IgE) antibodies that cause allergic reactions or disease.

35.2 Mechanism and classification of hypersensitivity reactions

Based on immune response and some miscellaneous factors, hypersensitivity reactions are classified as follows

Type I hypersensitivity or immediate hypersensitivity

It is characterized by the stimulation of helper T cells that are associated with production of IgE antibodies and inflammation. Type I is the most common hypersensitive reaction. **Atopy** or allergic reaction is the best example of type I reactions.

Type II hypersensitive disorders

This occurs due to activation of complement system by IgG and IgM antibodies. Some of these antibodies are specific for some antigens and the disease caused by such antibodies are called type II hypersensitive disorders such as **Graves' disease**.

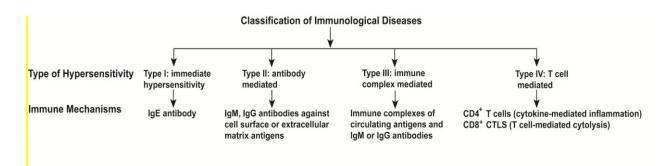**Type III hypersensitive disorders**

Various other antibodies make immune complexes in blood circulation and cause tissue damage. Such immune complex diseases are called type III hypersensitive disorders. **Arthus reaction** is a type III hypersensitive disorder.

Type IV hypersensitive disorder

It involves activation of phagocytes, T-lymphocytes, and natural killer cells. **Multiple sclerosis** is one of such kind.

In brief, majority of hypersensitive reactions are caused by stimulation of subset of T helper cells. They generally induce inflammation and tissue damage by recruiting neutrophils and macrophages.

Figure 35.1 Classification of hypersensitive reactions:

35.3 Diseases caused by antibodies

Disorders involving antibodies may occur either due to antigen antibody reaction or antigen-antibody complexes formed in various tissues. Haemolysis in transfusion reactions involves opsonization of cells by antibodies to stimulate the complement system. Further Phagocytes kill the opsonized cells leading to cell destruction. Sometimes antibody abnormalities may also cause conditions like Graves' disease. In other cases like antibody mediated glomerulonephritis, cell damage occurs because of leukocyte activation and inflammation.

Table 35.1 Diseases caused by antibodies:

Disease	Antigen	Mechanism
Autoimmune hemolytic anemia	Blood group antigens (Rh)	Phagocytosis of the red blood cells
Pemphigus vulgaris	Epidermal junction protein	Activation of protease and destruction of intercellular junction.
Myasthenia gravis	Acetylcholine receptor	Impaired nerve transmission.
Graves disease	Thyroid stimulating hormone (TSH) receptor	Stimulation of TSH leading to hyperthyroidism.
Insulin-resistant diabetes	Insulin receptor	Inhibition of insulin binding leading to hyperglycemia.
Pernicious anemia	Gut intrinsic factor	Decreased absorption of vitamin B12.
Goodpasture's syndrome	Non collagenous protein of kidney and lung	Inflammation leading to nephritis and hemorrhages in lungs.

35.4 Immune complex mediated diseases

Immune complex mediated diseases occur due to antibody reaction against either a self antigen or a non-self antigen. Such diseases are not restricted to a particular organ and are widely spread in the body. Serum sickness, Arthus reaction, polyarteritis nodosa, and poststreptococcal glomerulonephritis are some of the immune complex mediated diseases.

Serum sickness is an immunization of any individual with a non-self protein or foreign protein that leads to immune reaction.

Arthus reaction involves accumulation of antigen-antibody complexes in blood vessels.

Polyarteritis nodosa involves hepatitis B virus surface antigen which leads to vasculitis.

Poststreptococcal glomerulonephritis involves streptococcal cell wall antigen which leads to inflammation of kidney.

Immune complex mediated diseases may not be dangerous until the complexes are formed in very high amount and are not eliminated from the body. If such complexes tend to retain in blood vessels and capillaries, it may lead to their deposition followed by tissue injury.
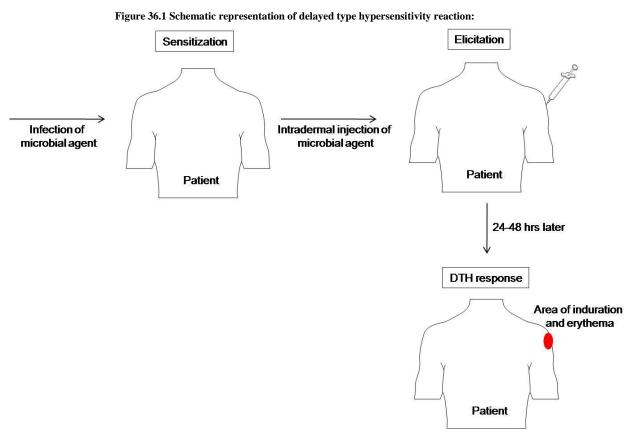
35.5 T lymphocyte mediated diseases

T lymphocyte damages the tissue either by direct killing of the target cells or by inducing inflammation. In some of the cases T cell kills the target cells bearing the MHC class-I molecules. Inflammation is mainly induced by the subset of T cells, namely Th1 and Th17.

Lecture 36: Hypersensitivity (Part II)

36.1 Delayed type hypersensitivity (DTH)

These types of reaction are caused by stimulation of CD4+ T cells and are mediated by cytokines. It is called delayed as it evolves 24 to 48 hrs after antigen reaction. In classical form of DTH, a guinea pig is first **sensitized** with the antigen and after 14 days challenged with the same antigen subcutaneously, the subsequent reaction is then analyzed in the **elicitation** reaction. In humans, most famous model is the injection of purified protein derivative (PPD) of *Mycobacterium tuberculosis* that elicits a DTH response in an individual who has been previously exposed to *M. tuberculosis* (tuberculin reaction).

Figure 36.1 Schematic representation of delayed type hypersensitivity reaction:

36.2 Other immunologic diseases

Systemic Lupus Erythematosus

Rheumatoid arthritis

Multiple sclerosis

Type I Diabetes Mellitus

Inflammatory bowel disease

36.2.1 Systemic Lupus Erythematosus

It is an autoimmune condition mostly affecting women. Common signs are body rashes, arthritis, haemolytic anemia, and CNS involvement. Presence of antinuclear antibodies (antibodies against double-stranded native DNA) is typical for systemic lupus erythematosus. Systemic lupus erythematosus is a complex disease condition where genetic and environment contributes to the outcome.

36.2.2 Rheumatoid arthritis

It is an inflammatory condition of joints, shoulders, elbows, fingers etc. Synovitis (inflammation of synovial membrane) occurs due to the immune response contributed both by cell mediated and humoral mediated immunity. Inflamed synovial fluid contains activated B-cells, macrophages and dendritic cells. Presence of cytokines in synovial fluid is seen that causes destruction of cartilage ligaments and tendons. **Cyclic citrullinated peptides** (CCP) are the antibodies that help in the diagnosis of the disease as they are typical for this condition. As role of cytokines and T-cells in this disease has been well established, specific molecules are being marked for this condition. Various therapeutic approaches are on the way but IL-1 antagonist and an antibody against the IL-6 receptor is the treatment of choice so far.

36.2.3 Multiple sclerosis

It is most prevalent in young adults characterized with neurologic symptoms especially inflammation of central nervous system and disorder in nerve conduction. Myelin acts as a self antigen and there is a regular lesion formation in the patient. B cell depletion is the approved treatment in some patients.

36.2.4 Type I Diabetes Mellitus

The disease occurs due to defective insulin production resulting in hyperglycemia (more glucose in blood) and ketoacidosis (high concentrations of ketone bodies in blood). In such individuals hormone replacement therapy is constantly required to compensate the deficiency of insulin in their body.

36.2.5 Inflammatory bowel disease

In this disease damage to intestinal wall occurs because of T-cell mediated inflammation. It occurs in two forms, namely **Crohn's disease** and **ulcerative colitis**. Therapeutic approaches include antibodies against p40 chain of IL-12 and IL-23, TNF and IL-17.

36.3 Immunologic diseases caused by cytotoxic T lymphocyte

Cytotoxic T lymphocyte (CTL) response against a viral infection can lead to injurious condition to the host cells and tissues. Many viruses produce a cytopathic effect which are detected by the CTLs and destroyed. While many viruses don't produce any cytopathic effects and CTLs may fail to distinguish it and destroy the cells. This often progresses towards a condition where the CTLs destroy the cells which may be harmful to the host. Development of myocarditis following the infection of Coxsackievirus B is a common consequence of this kind of reaction.

36.4 Therapeutic approaches for immunologic diseases

The field of immunology has grown up to a large extent due to increasing research and understanding of the basic science. The novel therapeutics, have greatly increased the life expectancy of the human population. Anti-inflammatory drugs like corticosteroids are used to treat the hypersensitivity diseases. Many cytokines are also used in controlling the chronic inflammation and tissue damages of same origin.

Lecture 37: Congenital and acquired immunodeficiency (Part I)

37.1 General features of immunodeficiency diseases

Some of the important characteristics of immunodeficiency disorders are

1) One of the main aftermaths of immunodeficiency is the increased sensitivity towards the infection. Due to lack of sufficient humoral and cell mediated immunity there is an increased risk to infection by pus-forming bacteria, viruses and other intra and intercellular microbes. State of immunodeficiency leads the subject to a compromised state wherein an infected body shows less to no resistance against the foreign pathogen. In such cases opportunistic infections are much probable which otherwise a healthy patient would easily get rid of.

2) Risk to certain types of cancer increases in immuno-deficient patients. Oncogenic viruses are the ones which can cause cancer e.g. Epstein-Barr virus. This may occur because of T- cell deficiency as they play an important role in defense mechanism of the body.

3) Autoimmunity is another factor related with some immunodeficiencies. In autoimmune condition body cannot distinguish between self and nonself and produces an aberrant immune response.

4) Sometimes immunodeficiency may occur as a consequence of faulty mechanisms of adaptive and innate immunity or due to some error in lymphocyte development.

37.2 Congenital (Primary) immunodeficiencies

In congenital or primary immunodeficiency, the cause may be a genetic disorder or in other words innate immune response. In general, complement pathway gets affected due to the genetic abnormalities. Further, lymphocyte development gets altered by mutation in genes that code for a particular molecule e.g. enzymes, transcription factors, adaptors etc. Thus a defective innate immune system may not lead to a proper antigenic stimulation because undeveloped B-lymphocytes may not produce sufficient antibodies. This can be further confirmed by low levels of serum immunoglobulin (Ig), poor antibody response to vaccines, and decreased number of B-cells in the circulation. Similarly, undeveloped T-lymphocytes may hamper cell mediated immunity. Confirmation of reduced T-cell response can be done by deficient cutaneous delayed type

hypersensitivity reactions to antigens and low proliferative response of blood lymphocytes to polyclonal T cell activators.

37.2.1 Defects in innate immunity

Usually defects in innate immunity results from inherited abnormalities in complement and phagocytes. The disorder in complement system and phagocytes results in repeated infections as it is related to inherited genes.

Table 37.1 List of congenital defects involving innate immune system:

Disease	Functional Deficiencies
Defects in Toll-like receptor (TLR) signaling	-Frequent infections due to defects in TLR and CD40 signaling. -Defects in the production of type I interferon.
Chronic granulomatous disease	-Defect in the production of reactive oxygen species by scavenger cells.
Leukocyte adhesion deficiency type 1	-Defective leukocyte adhesion and migration because of decreased or absence of β_2 integrins expression.
Leukocyte adhesion deficiency type 2	-Defective leukocyte rolling and migration due to decrease or loss of expression of E- and P-selectins.
Leukocyte adhesion deficiency type 3	-Defective leukocyte adhesion and migration linked to defective signaling.
Chédiak-Higashi syndrome	-Defective vesicle fusion and lysosomal function in many cell types including macrophages, neutrophils, natural killer cells, dendritic cells, and cytotoxic T cells.

37.2.2 Defective microbicidal activities of phagocytes

   I.   Chronic granulomatous disease
  II.   Leukocyte adhesion deficiencies
 III.   Defects in natural killer cells and other leukocytes
  IV.   Inherited defects in TLR pathways, nuclear factor kB signaling, and type I interferons.
   V.   Defects in the IL-12/IFN –γ pathway

I. **Chronic granulomatous disease**

It is a very rare condition that leads to faulty production of superoxide anion, one of the reactive oxygen species that establishes a major microbicidal mechanism of phagocytes. The actual cause of the disease is due to the alteration in the building blocks of phagocyte oxidase enzyme complex.

II. **Leukocyte adhesion deficiencies**

This occurs because of the abnormalities in leukocyte and endothelial adhesion molecules. It has three forms. Generally in this condition, abnormality is seen in neutrophils recruitment as they form the first line of cellular defense.

III. **Defects in natural killer cells and other leukocytes**

The Chediak-Higashi syndrome – it is an autosomal recessive condition characterized by leucopenia and such cells are also faulty in chemotaxis and phagocytosis.

IV. **Inherited defects in TLR pathways, nuclear factor kB signaling, and type I interferons**

Genetic disorder of this type involves the MyD88 adaptor and the IRAK-4 and IRAK-1 kinases. The mechanism leads to the induction of proinflammatory cytokines and different forms of mutation.

V. **Defects in the IL-12/IFN –γ pathway**

Dendritic cells and macrophages secrete IL-12 and further interferon-γ by cytotoxic T cells. Any mutation in the genes encoding IL-12 leads to deficient cytotoxic T cell response and interferon-γ production.

37.2.3 Severe combined immunodeficiencies (SCID)

Those genetic abnormalities that affect both cell mediated and antibody mediated immunity are known as combined immunodeficiencies and a part of these in which most of the T-cells are lost or defective are known as severe combined immunodeficiencies (SCID). The main culprit for SCID is the malfunctioned T-lymphocyte development, it has severe forms namely

Adenosine deaminase (ADA) deficiency- ADA deficiency is caused by mutation in the gene encoding ADA protein. It leads to accumulation of toxic metabolites in the

lymphocytes. The Condition causes progressive decrease in T, B and NK cells in the blood circulation and also decreased level of serum immunoglobulins.
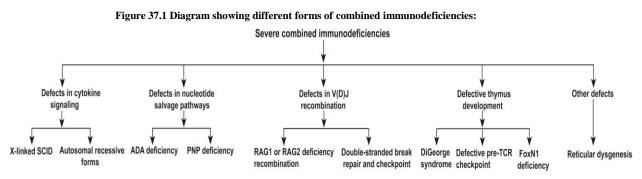
Omenn's syndrome- This is caused by defective recombination during the development of T and B lymphocytes. The condition generally leads to SCID due to malformed lymphocytes population.

Purine nucleoside phosphorylase (PNP) deficiency- These are enzymes involved in purine catabolism. It leads to accumulation of toxic metabolites in the lymphocytes and cause the condition similar to ADA deficiency.

X-linked SCID- The defect is caused by mutations, in the γ chain of the cytokine receptors that leads to defective T cell development. Condition is characterized by decreased T cell and immunoglobulin in the serum.

The Bare lymphocyte syndrome- Deficiency of major histocompatibility class II molecules due to chromosomal abnormalities leads to this condition.

DiGeorge syndrome - Related to defective thymic development in children.

Figure 37.1 Diagram showing different forms of combined immunodeficiencies:

ADA- Adenosine deaminase

PNP- Purine nucleoside phosphorylase

RAG- Recombination activating gene

FoxN1- Forkhead box protein N1

37.2.4 Defects in T-lymphocyte activation and function

Recently defects in T-lymphocyte activation and function have played a role of an eye-opener as their molecular studies are being carried out in different parts of the world. Many studies indicate that defects in T-cell protein itself have created various mutations in the genes. Some of the examples are defective TCR mediated signaling due to mutations in the ZAP70 gene and defective transcription factors leading to decreased cytokine synthesis. **Wiskott-Aldrich syndrome** is one of the diseases that occur due to

faulty T-cell activation. The gene responsible for this condition encodes a cytoplasmic protein called WASP (Wiskott-Aldrich syndrome protein).

37.2.5 Ataxia-Telangiectasia

It is one of the inherited abnormalities characterized by ataxia, neurological disorders, vascular malformation and multiple tumors. In this condition both B and T cells get affected and the condition is caused by a gene that encodes a protein called ATM (**Ataxia –Telangiectasia mutated**).

37.3 Therapeutic approaches for congenital immunodeficiencies

Till date hematopoietic stem cell transplantation has been approved to be the treatment of choice for many immunodeficiency diseases including SCID with ADA deficiency, bare lymphocyte syndrome and leukocyte adhesion deficiencies. Enzyme replacement therapy too has proved a success in some ADA and PNP deficient cases. Non-technically therapy of choice for such disorders would be gene therapy i.e. to replace the mutated gene in self-renewing stem cells. However some patients undergoing such treatment have developed leukemia therefore gene therapy has certain limitations to cure such diseases.

Lecture 38: Congenital and acquired immunodeficiency (Part II)

38.1 Acquired immunodeficiencies

Acquired immunodeficiencies are the ones that are not inherited but are acquired during lifetime. These may occur either as a biological complication of another disease or the diseases that develop due to complications of therapies for other diseases. Malnutrition, neoplasms, and infections are some of the conditions in which immunodeficiency acts as a complicating element. Other form of acquired immunodeficiency occurs in patients where spleen is absent or has been removed surgically.

38.1.1 HIV/AIDS

It is caused by human immunodeficiency virus (HIV) infection and is associated with opportunistic infections, malignant tumors and immunosuppression. It affects the immune system by killing dendritic cells, CD4+ helper T cells, and macrophages.

38.1.2 HIV structure and genes

HIV is a lentivirus belonging to the family *Retroviridae*. It infects macrophages, dendritic cells and CD4 T cells leading to immunosuppression. The structure of HIV virion consists of two copies of positive single stranded RNA, viral proteins and phospholipids. The size of RNA genome is 9.2kb in length with 3 important genes in addition to others. These three genes namely gag, pol, and env have specific roles to play in the pathogenicity of HIV. Gag encodes structural proteins, env encodes envelope glycoproteins gp120 and gp41 for infection, and pol encodes reverse transcriptase, integrase and viral protease enzymes needed for virus replication.

38.1.3 Viral life cycle

HIV gets transmitted from one person to the other through three possible ways.

1) Sexual contact
2) Blood transfusion or through milk
3) Transplacental passage

CXCR4 and CCR5 are the chemokine receptors that act as co-receptors for HIV. CD4+ T-cells are the cells of choice for HIV replication. Once inside the cell, HIV exists in a provirus form after integration of viral DNA with the host cell genome. Long terminal

repeats control the transcription of the provirus and full length viral transcripts are produced and expressed as proteins followed by the formation of an infectious virion.

38.1.4 Clinical course of HIV infection

It is divided in different phases based on the quantity of virus in the patient's plasma and CD4+ T cell count in the blood.

1) Acute phase

    It begins after three to six weeks of infection. Acute phase is characterized by viremia and initial reduction in CD4+ T cell count while the T cell count may gradually return to normal.

2) Chronic phase

    It may last for many years and the virus is held within the lymphoid tissue. Patients do not show any symptoms or may suffer from some minor infection.

3) Lethal phase

    This phase is reached when the blood **CD4+ T cell count drops below 200 cells /mm3**. This stage is known as AIDS and is characterized by immunosuppression and opportunistic infections.

38.1.5 Elite controllers and long-term nonprogressors

Most of HIV sufferers gradually develop AIDS but approximately 1% of infected population does not develop the disease. These patients so called Elite controllers have persistent viremia and high count of CD4+ and CD8+ T cell but no disease for at least ten to fifteen years. Prevention of AIDS in the elite controllers has been linked to genetics of the individual but the actual factors are yet to come out.
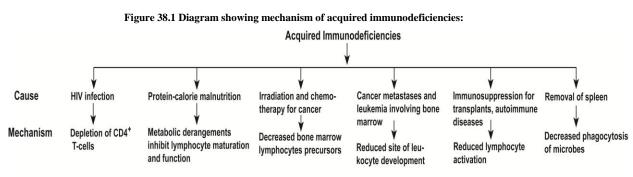
38.2 Pathogenesis of HIV infection and AIDS

HIV infection begins as an acute form and with slow progression changes to chronic form. Memory CD4+ T cells get infected and there is depletion of lymphocytes. Progression of disease from acute to chronic form is indicated by dissemination of the virus, viremia and the development of host immune responses. Besides CD4+ T cells, macrophages and dendritic cells also get affected. In later stages, lymph nodes and spleen become the targeted replication sites for the virus.

38.3 Mechanisms of immunodeficiency caused by HIV

Obviously HIV impairs both adaptive and innate immune system but cell mediated immunity gets affected markedly. Diminishing of CD4+ T cells in HIV infected patients is the result of cytopathic effect of the virus. The lysis of CD4+ T cells may occur due to the increased plasma membrane permeability and the flow of lethal amounts of calcium leading to apoptosis. Possibly it may also happen that viral production hampers cellular protein synthesis thereby causing cell death. Other reasons of reduction in lymphocytes would be loss of function of CD4+ T cells in the infected patients.

38.4 Mechanism of immune evasion by HIV

HIV is a dreaded disease. It has a high mutation rate because of its genome content and thus it may evade any antibody response. MHC class I molecules may have some role in its immunity which can be confirmed by evasion of CTLs through down-regulation of MHC I molecules.

Figure 38.1 Diagram showing mechanism of acquired immunodeficiencies:

38.5 Treatment and prevention of AIDS and vaccine development

Currently three classes of antiviral drugs are administered to the human patients.

The first class is the nucleoside analogues that inhibit reverse transcriptase activity such as 3'-azido-3'deoxythymididne (AZT), deoxycytidine analogues and deoxyadenosine analogues. These drugs may help in decreasing the levels of HIV genomic RNA in plasma for several months to years. It cannot slow down the progression of disease because the newly formed viruses with mutated version show resistance to these drugs. Non-nucleoside reverse transcriptase inhibitors and viral protease inhibitors have also been developed to combat the disease. HAART (highly active antiretroviral therapy) or ART (antiretroviral therapy) has emerged as one of the most effective triple-drug therapy

as it has shown promising results by reducing the plasma viral RNA to undetectable level even up to three years.

Development of effective vaccine against HIV is on the way as it sets a priority for biomedical researchers worldwide. HIV virus has a tendency to mutate therefore construction of an effective HIV vaccine is a challenging task. Vaccines for simian immunodeficiency virus (SIV) are already out in the market and the results are encouraging. SIV is similar to HIV in terms of its infectivity to macaques that is similar to AIDS in humans. Attempts to construct a vaccine that can generate both humoral as well as antibody mediated immunity may prove a better option for HIV.

Lecture 39: Laboratory techniques commonly used in immunology (Part I)

Many immunological techniques are used regularly in the laboratories to assess the immune status of the individual or to diagnose the diseases. Generally all or most of these techniques require antibodies to complete the routine procedure. The specificity of an antibody for a particular antigen makes it a valuable tool for diagnostics and many other molecular biological techniques. The discovery and uses of monoclonal antibodies have revolutionized our current understanding of immunology.

39.1 Immunoassays

Quantification of the antigen is a valuable data in both basic and clinical research sciences. Immunochemical methods of antigen quantitation are usually based on reactivity of pure antigen and antibody with an indicator molecule. When the antigen or antibody is labeled with a radioisotope it can be quantified by the decay of radioactive element by the assay called **Radioimmunoassay (RIA)**. When the antigen or antibody binds with an enzyme, it can be quantified by spectrophotometer reading upon conversion of substrate into a color by the enzyme, the assay is called an **enzyme-linked immunosorbent assay (ELISA)**. Many forms of RIA and ELISA exist but the most commonly used one is the sandwich method in which two different forms of antibodies are used against different areas of an antigen. Briefly, a known quantity of antibody is attached to the solid support in a microtiter plate. The antigens to be tested are used in different concentration along with a known concentration of control antigens and are allowed to react. The unbound antigens are washed with the appropriate solution and an enzyme linked or radiolabeled second antibody is added to react with the complex. The results from the standard solutions are used to construct the standard curve and accordingly the quantification of the test antigen is completed.
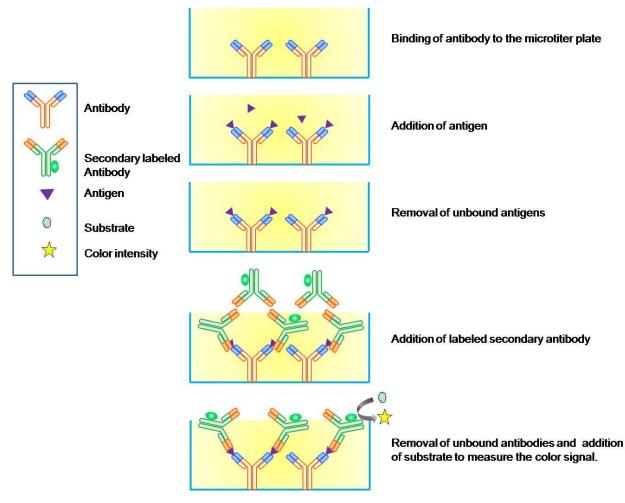
Figure 39.1 Schematic representation of an enzyme-linked immunosorbent assay:

39.2 Identification and purification of proteins

Antibodies can be used to identify and purify specific proteins from a mixture. Two most commonly used methods are Immunoprecipitation and immuno-affinity chromatography. Western blot is another commonly used technique to determine the presence and size of a specific protein.

39.2.1 Immunoprecipitation assay:

Immunoprecipitation is a method in which a specific antigen is pooled from a mixture by using a specific antibody. The antibody is added to a mixture of antigen along with staphylococcal protein A or G. The Fab portion of the antibody binds to the antigen or protein and Fc protein of the same antibody is captured by staphylococcal protein A or G beads. Unbound antibodies are removed by washing the beads with detergents. Specific

antigens or protein bound with the antibodies are detached with the help of a strong denaturating agent (sodium dodecyl sulfate) and separated using a sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) gel.
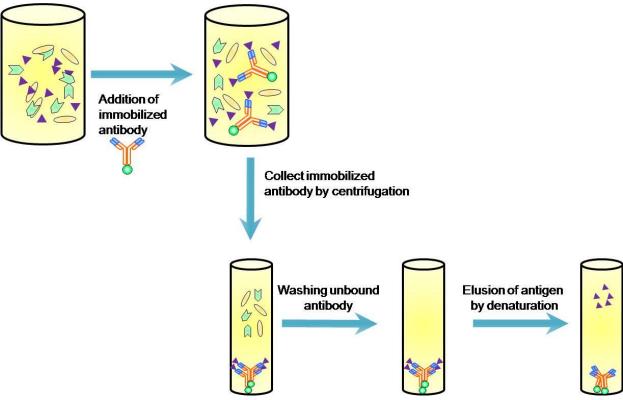
Figure 39.2 Schematic representation of immunoprecipitation assay:

39.2.2 Immuno-affinity chromatography:

Immuno-affinity chromatography is a method in which specific antibodies are bound to an insoluble support. A mixture of antigens is allowed to pass through the column that contains the bound antibodies. The antigens bind to the antibodies based on their specificities and the unbound ones are washed out. The bound antigens are eluted form a specific antibody by changing the pH or high salt concentration which breaks the bond between antigen and antibodies. The same method can be used to identify the specific antibody by binding the antigen to an insoluble support. It is a useful technique to identify and purify the specific antigen or antibody from a sample.
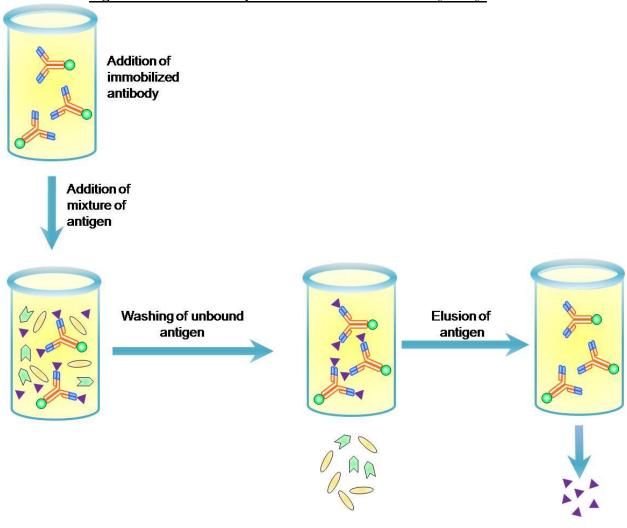
Figure 39.3 Schematic representation of immuno-affinity assay:

Lecture 40: Laboratory techniques commonly used in immunology (Part II)

40.1 Western Blotting Assay

It is the most popular and widely used method to identify and determine the size of an unknown protein. The mixtures of protein are first subjected to an SDS-PAGE, so that the proteins can be separated based on their size. The gel is then transferred in to a nitrocellulose membrane in a way to represent its exact replica. Protein band on the membrane is then detected by binding an antibody specific to that protein followed by a labeled secondary antibody which binds to the first antibody. The second antibody is labeled with an enzyme that generates chemiluminescent signals which can be detected over the x-ray film. The sensitivity and specificity of western blot can be increased by purifying the proteins first with Immunoprecipitation assay.

<u>*Interesting Facts</u>

The scientist who first blotted the DNA over the membrane by capillary transfer was Edwin Southern, and hence named **Southern blotting**. By analogy, transfer of RNA over the membrane is called **Northern blotting** and transfer of protein is called **Western blotting**.
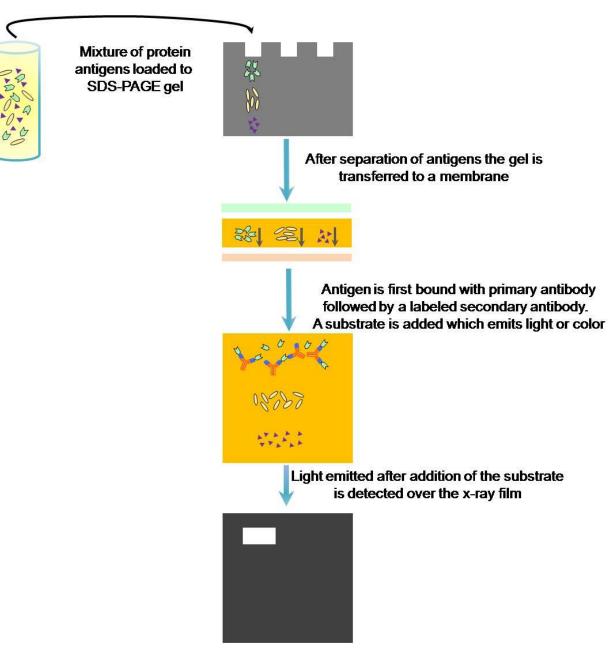
There is no Eastern blot???

Figure 39.1 Schematic representation of western blotting assay:

Mixture of protein antigens loaded to SDS-PAGE gel

After separation of antigens the gel is transferred to a membrane

Antigen is first bound with primary antibody followed by a labeled secondary antibody. A substrate is added which emits light or color

Light emitted after addition of the substrate is detected over the x-ray film

40.2 Labeling of cells

There are certain antigens specific for particular cell types. Antibodies against those antigens are used to identify the cells and tissues from a mixed population. The antibodies are labeled with fluorochrome, radioisotopes, or enzymes in order for their detection through different sensitive detector system.

40.2.1 Flow Cytometry

The cell lineages, stages of maturation, and types can often be determined by expression of molecules over the cell surface. The method generally involves staining the cells with fluorescence dye specific against the expressed molecules over the cell surface. The surface molecules are often termed as **markers** or "**cluster of differentiation**" (**CD**). The flow cytometer is a specific instrument which can detect the number of cells expressing the molecules based on the fluorescence detected by sensors. Occasionally cytoplasmic molecules are stained by fluorochrome- labeled antibody after permeabilizing the cells which permits the entry of labeled antibodies. DNA can also be labeled with **propidium iodide** to study the cell cycle. The dying cells can be labeled with **annexin-V** which binds with *phosphatidylserine* expressed in the cells undergoing apoptosis. It is a powerful tool to analyze the cell types and the cells which express different epitopes.

A more advanced technique called fluorescent- activated cell sorter is used to separate different cell types based on the intensity of the fluorescence on electro-magnetic field.
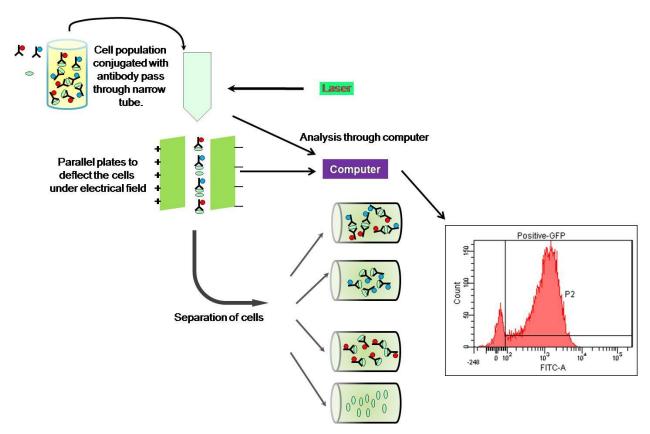
Figure 39.1 Schematic representation of fluorescent- activated cell sorter assay:

40.2.2 Immunofluorescence and immunohistochemistry

Antibodies can be used to visualize the presence of antigen within the cell and tissue. A conventional way to localize the antibody is by immunoperoxidase technique using horseradish peroxidase or alkaline phosphatase. In immunofluorescence the fluorescence labeled antibody is incubated with the cells or tissue and are visualized under the immunofluorescence microscope. More advanced version of the immunofluorescence microscope is confocal microscopy where details of the cells can be visualized by more sophisticated laser technology.

40.3 Studying T cell response

T cell activation depends on the stimuli or the external antigen presented to it through an antigen presenting cell. Many carbohydrate binding lectins such as concanavalin-A and phytohemagglutinin are used to activate T cell as a **polyclonal activator**. Polyclonal activation is done in order to activate the T cells against an unknown antigen. T cells can also be activated by pharmacological reagents such as phorbol ester and ionophores. This

method is useful in studying the antigen activated T cells that are previously primed with a different antigen.

40.4 Studying B cell response

Polyclonal activation of B cell is difficult as only few cells are specific against an antigen during the time of clonal selection. Generally anti-Ig antibodies are used for polyclonal activation of B lymphocytes. Anti-Ig antibodies bind to the constant region of the membrane bound Ig on the B cell which will have the same effect as of the antigen binding to the hypervariable region of the Ig.